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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: W. James Jackson

Serial No.: 09/677,752 Group Art Unit: 1645

Filed: October 3, 2000 Examiner: V. Ford

For: CHLAMYDIA PROTEIN, GENE SEQUENCE AND USES THEREOF Attorney Docket No.: 7969-087-999

DECLARATION UNDER 37 C.F.R. §1.131 OF DR. W. JAMES JACKSON

Assistant Commissioner for Patents
U.S. Patent and Trademark Office
P.O. Box 2327
Arlington, VA 22202

Sir:

I, Dr. W. James Jackson, declare and state the following:

1. I am the sole inventor of the subject matter claimed in the above-identified application.

2. I have carefully reviewed the application including the claims as amended by the Response Under 37 C.F.R. §1.111 with Amendments being submitted with this declaration.

3. I understand that this declaration is filed to show facts to demonstrate that prior to June 15, 2000, I had conceived the subject matter of pending Claims 15-24, 31-32 and 41 directed to a vaccine composition, against *Chlamydia* infection, comprising the isolated or recombinant putative membrane protein E (PMPE) polypeptide of *Chlamydia* having a molecular weight between 90 and 115kDa and had reduced to practice the isolated or recombinant PMPE.

4. Attached as Exhibits 1-12C are the following documents:

a. Exhibits 1-3: a copy of three memo Requests to Research Genetics from me requesting synthesis of primers to be used to obtain the nucleic acid encoding PMPE by PCR.

b. Exhibit 4 is a copy of pages 8, 9 and 10 of the Laboratory Notebook of Kelly Johnson. In particular, page 9 relates to the creation of PCR products to isolate the *Chlamydia trachomatis* nucleic acid encoding PMPE using the primers requested by me in the documents designated Exhibit 1.

c. Exhibit 5 is a copy of pages 20 and 21 of the Laboratory Notebook of Kelly Johnson. These pages relate to creation of PCR products comprising isolated *Chlamydia trachomatis* nucleic acid encoding PMPE using the primers requested by me in the documents designated Exhibit 2.

d. Exhibit 6 is a copy of page 41 of the Laboratory Notebook of Kelly Johnson relating to PCR screen for colonies that transformed with *C. trachomatis* nucleic acid encoding PMPE (Ct pmpE-pQE-M15).

e. Exhibit 7 is a copy of page 42 of the Laboratory Notebook of Kelly Johnson relating to Expression Analysis of the cloned PMPE. Results of the Western Blot Analysis are shown.

f. Exhibit 8 is a copy of page 65-68. of the Laboratory Notebook of Kelly Johnson relating to production of plasmid preparations for expression of PMPE.

g. Exhibit 9 is a copy of pages 67 and 68 of the Laboratory Notebook of Kelly Johnson relating to sequencing of the recombinantly expressed PMPE.

h. Exhibit 10 is a copy of a five page Monthly Report relating to *Chlamydia spp.* Project Number: 0120 and 0125. In particular, pages 4-5 of the Monthly Report relate to a summary of the cloning and expression of *Chlamydia trachomatis* PMPE.

i. Exhibit 11 is a copy of two pages of a Monthly Report relating to *Chlamydia spp.* Project Number: 0120 and 0125. In particular, page 2, of the report indicates that *Chlamydia* PMPE protein was cloned and expressed.

j. Exhibit 12 is a copy of three pages, designated 12-A; 12-B; and 12-C of a multi-page Quarterly Review relating to *Chlamydia trachomatis* Vaccine Development.

    i. Exhibit 12-A is a copy of a page of the multi-page Quarterly Review (review) relating to the objectives for identification and evaluation of proteins like High Molecular Weight (HMW) protein of *Chlamydia* identified by Antex Biologics Inc. as an immunogenic protein suitable for a vaccine composition.

    ii. Exhibit 12-B is a copy of a page of the multi-page Review relating to sequence identity analysis to determine which PMP proteins are homologous to HMW. As indicated, PMPE (designated PMPe: pro or PMP: 5) is most closely related to HMW.

iii. Exhibit 12-C is a copy of a page of the multi-page Review summarizing data related to production of PMPE.

5. I have reviewed each of the documents in Exhibits 1-12C. Although the dates have been redacted, each of the dates of the documents is prior to June 15, 2000. I confirm that the work evidenced by the documents and all act relied upon in this declaration were carried out in the United States of America prior to June 15, 2000.

6. It should be noted that confidential information of Antex Biologics Inc. (Antex), Assignee of the present application, present in each of the documents not relating to PMPE has been redacted. It is my understanding that none of the material redacted which is not relevant to PMPE is necessary to understand the remaining information and its omission does not make the remaining information misleading.

7. I authored the documents designated Exhibits 1-3 and 10-11 and 12-A, 12-B and 12-C. The hand written notes on Exhibit 12-B were also authored by me. The experimental work reflected in Exhibits 4-9 was performed at my direction and under my supervision and control by Kelly Johnson, an employee of Antex my employer, the Assignee of the present application. The pages of the Laboratory Notebook are in the handwriting of Kelly Johnson.

8. Prior to June 15, 2000, as evidenced by Exhibits 1-11, I directed the successful cloning, expression and isolation of PMPE of *Chlamydia* of approximate molecular weight between 90 and 115 kDa.

9. Prior to June 15, 2000, as evidenced by Exhibits 12-A through 12-C, I appreciated that a composition comprising isolated PMPE of *Chlamydia* of molecular weight between 90 and 115 kDa, would be suitable for use as a vaccine c mposition. In part, this appreciation was based upon my knowledge of the suitability of another protein, isolated and

cloned from *Chlamydia*, by me and another co-inventor, at Antex, *i.e.* an outer membrane protein of about 105-115 kDa designated High Molecular Weight (HMW) protein (or HMWP). Accordingly, prior to June 15, 2000, I conceived the idea of a vaccine composition for use against *Chlamydia* infection, in which the vaccine composition comprises an isolated or recombinant *Chlamydia* PMPE of between 90 and 115 kDa.

I hereby declare further that all statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true and further I make these statements with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Respectfully submitted,

Date: 11/30/01

By: _____

W. James Jackson, Ph.D.

EXHIBIT 1

To: Kim
c/o Research Genetics
Huntsville, Al 35801

Date:

From: Jim Jackson
Antex Biologics (formerly MicroCarb Inc.)
Gaithersburg, MD 20879
301-590-0129

Subject: Oligonucleotide Synthesis

Please synthesize the following nine (9) oligonucleotides at the 40 nM scale, Trityl-off auto. No HPLC purification is necessary.

Ct-pmpE-Fm/p-Nde
5'- AGG CAG AGG CTC GAG **ATG AAA AAA GCG TTT TTC TTT TTC CTT ATC GG** - 3' 47 mer

Ct-pmpE-RCf/p-Xho
5' - AGG CAG AGG CTC GAG **GAA TCG CAG AGC AAT TTC CCC ATT GAG** - 3' 42 mer

Ct-pmpE-Fm/t-RI
5'- AGG CAG AGG GAA TTC **ATG AAA AAA GCG TTT TTC TTT TTC CTT ATC GG** - 3' 47 mer

Ct-pmpE-RCh/t-Sal
5' - AGG CAG AGG GTC GAC TTA ATG GTG ATG GTG ATG GTG GAA **TCG CAG AGC AAT TTC CCC ATT GAG** - 3' 63 mer

Ct-pmpF-Fm/p-Nde
5'- AGG CAG AGG CTC GAG **ATG ATT AAA AGA ACT TCT CTA TCC TTT GC** - 3' 44 mer

EXHIBIT 2

To: Kim
c/o Research Genetics
Huntsville, Al 35801

Date:

From: Jim Jackson
Antex Biologics (formerly MicroCarb Inc.)
Gaithersburg, MD 20879
301-590-0129

Subject: Oligonucleotide Synthesis

Please synthesize the following six (6) oligonucleotides at the 40 nM scale, Trityl-off auto. No HPLC purification is necessary.

L2-pmpE-Fm/28a-Nco*

5' - ATC CAG CAG AGG CC ATG GAA AAA GCG TTT TTC TTT TTC CTT A - 3' 42 mer

L2-pmpE-RCf/28a-Sal

5' - ATC CAG CAG AGG GTC GAC GGC C GAA TCG CAG AGC AAT TTC CCC ATT GA - 3' 48 mer

L2-pmpEsdm-Nde-F

5' - CGA GAA AAT CAT CCT GGA TTC CAC ATG CGC TCT TCC GGA TAC TCT GCG G- 3' 49 mer

L2-pmpEsdm-Nde-RC

5' - CCG CAG AGT ATC CGG AGG AGC GCA TGT GGA ATC CAG GAT GAT TTT CTC G - 3' 49 mer

L2-pmpEsdm-Xba-F

5' - GGA CTA GCT AGA GAG GTT CCT TCC AGA ATC TTT CTT ATG CCC AAC TCA G - 3'

49 mer

L2-pmpEsdm-Xba-RC

5' - CTG AGT TGG GCA TAA GAA AGA TTC TGG AAG GAA CCT CTC TAG CTA GTC C - 3'

49 mer

The purchase order number for this synthesis is 99-0016.

Please call if you require any additional information. I look forward to receiving these materials.

EXHIBIT 3

To: Kim
c/o Research Genetics
Huntsville, Al 35801

Date:

From: Jim Jackson
Antex Biologics (formerly MicroCarb Inc.)
Gaithersburg, MD 20879
301-590-0129

Subject: Oligonucleotide Synthesis

Please synthesize the following six (6) oligonucleotides at the 40 nM scale, Trityl-off auto. No HPLC purification is necessary.

Ct-pmpE-Fn/p-Nde2
5'- ATC CAG CAG AGG CAT ATG **AAA AAA GCG TTT TTC TTT TTC CTT ATC GG** - 3' 47 mer

Ct-pmpE-Fn/t-RI2
5'-ATC CAG CAG AGG GAA TTC **ATG AAA AAA GCG TTT TTC TTT TTC CTT ATC GG** - 3' 50 mer

Ct-pmpF-Fn/p-Nde2
5'-ATC CAG CAG AGG CAT ATG **ATT AAA AGA ACT TCT CTA TCC TTT GC** - 3' 44 mer

Ct-pmpF-Fn/t-RI2
5'-ATC CAG CAG AGG GAA TTC **ATG ATT AAA AGA ACT TCT CTA TCC TTT GC** - 3' 47 mer

Cp-pmp13-Fm/30f-Sal
5'- ATC CAG CAG AGG GTC GAC **GAG AAC TTT GAT GGA TCG AGT GGG AA** - 3' 44 mer

EXHIBIT 4

PROJECT PCR product generation C.p. & C.t. Notebook No. _____ Continued From Page _____

PURPOSE: The purpose of this experiment is to create PCR products using Chlamydia trachomatis and Chlamydia pneumoniae genomic DNA with pmp and H47 primers designed by Dr. Jackson

MATERIALS/METHODS: The methods described on page 1 will be used here as well. 1 µl of genomic C.p DNA (prepared by Andrea Harris) and 4 µl of genomic C.t DNA (prepared by Jim Jackson) will be used in this experiment. The following sets of primers will be used:

G9070879 CpH47-RCf/p-Sal 38mer
5'-AGGCAGAGGGTCGACTTCTTCAGGTTTCAGGGCAATGA-3'
641 ug Unpurified and Lyophilized
pET24b MW = 11762 ug/umol pmol/ug = 85 %GC = 52.6 Tm = 116°C

G9070880 CpH47-Fm/b-Nco 43mer
5'-AGGCAGAGGCCATGGAGGGGAAAAAAGAATCTCGAGTTTCCGA-3'
801 ug Unpurified and Lyophilized
pBAD MW = 13382 ug/umol pmol/ug = 75 %GC = 51.2 Tm = 130°C

G9070881 CpH47-RCf/b-Xba 40mer
5'-AGGCAGAGGTCTAGATGTTCTTCAGGTTTCAGGGCAATGA-3'
810 ug Unpurified and Lyophilized
pBAD MW = 12392 ug/umol pmol/ug = 81 %GC = 47.5 Tm = 118°C

G9070882 CpH47-Fn/t-RI 38mer
5'-AGGCAGAGGGAATTCATGATAACTAAGCAATTGCGTTC-3'
778 ug Unpurified and Lyophilized
pTRE MW = 11738 ug/umol pmol/ug = 85 %GC = 42.1 Tm = 108°C

G9070883 CpH47-RCh/t-Sal 59mer
5'-AGGCAGAGGGTCGACTTAATGGTGATGGTGATGGTGTTCTTCAGGTTTCA GGGCAATGA-3'
696 ug Unpurified and Lyophilized
pTRE MW = 18390 ug/umol pmol/ug = 54 %GC = 49.2 Tm = 176°C

G9070884 CpH47-Fm/p-Nde 35mer
5'-AGGCAGAGGCATATGATAACTAAGCAATTGCGTTC-3'
743 ug Unpurified and Lyophilized
pET21b MW = 10794 ug/umol pmol/ug = 93 %GC = 42.9 Tm = 100°C

G9070885 CpH47-Fm/q-Bam 41mer
5'-AGGCAGAGGGGATCCGGGAAAAAAGAATCTCGAGTTTCCGA-3'
592 ug Unpurified and Lyophilized
pQE30 MW = 12741 ug/umol pmol/ug = 78 %GC = 51.2 Tm = 124°C

G9070886 CpH47-RCs/q-Sal 41mer
5'-AGGCAGAGGGTCGACTTATTCTTCAGGTTTCAGGGCAATGA-3'
618 ug Unpurified and Lyophilized
pQE30 MW = 12680 ug/umol pmol/ug = 79 %GC = 48.8 Tm = 122°C

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Kelly Johnson — Signed — Date

Andrea M Harris — Signed — Date

K9110131 Ct-pmpE-Fm/p-Nde 47mer
5'-AGGCAGAGGCTCGAGATGAAAAAAGCGTTTTTCTTTTTCCTTATCGG-3'
717 ug Unpurified and Lyophilized
MW = 14477 ug/umol pmol/ug = 69 %GC = 42.6 Tm = 134°C
Notes: pET24b

K9110132 Ct-pmpE-RCf/p-Xho 42mer
5'-AGGCAGAGGCTCGAGGAATCGCAGAGCAATTTCCCCATTGAG-3'
877 ug Unpurified and Lyophilized
MW = 12956 ug/umol pmol/ug = 77 %GC = 54.8 Tm = 130°C
Notes: pET24b

K9110133 Ct-pmpE-Fn/t-RI 47mer
5'-AGGCAGAGGGAATTCATGAAAAAAGCGTTTTTCTTTTTCCTTATCGG-3'
911 ug Unpurified and Lyophilized
MW = 14476 ug/umol pmol/ug = 69 %GC = 38.3 Tm = 130°C
Notes: pTLZ

K9110134 Ct-pmpE-RCh/t-Sal 63mer
5'-AGGCAGAGGGTCGACTTAATGGTGATGGTGATGGTGGAATCGCAGAGCAA
TTTCCCCATTGAG-3'
1300 ug Unpurified and Lyophilized
MW = 19585 ug/umol pmol/ug = 51 %GC = 50.8 Tm = 190°C
Notes: pTLZ

K9110135 Ct-pmpF-Fm/p-Nde 44mer
5'-AGGCAGAGGCTCGAGATGATTAAAAGAACTTCTCTATCCTTTGC-3'
660 ug Unpurified and Lyophilized
MW = 13512 ug/umol pmol/ug = 74 %GC = 43.2 Tm = 126°C
Notes: pET24b

K9110136 Ct-pmpF-RCf/p-Xho 41mer
5'-AGGCAGAGGCTCGAGAAAGACCAGAGCTCCTCCTGCATTGA-3'
660 ug Unpurified and Lyophilized
MW = 12613 ug/umol pmol/ug = 79 %GC = 56.1 Tm = 128°C
Notes: pET24b

K9110137 Ct-pmpF-Fn/t-RI 44mer
5'-AGGCAGAGGGAATTCATGATTAAAAGAACTTCTCTATCCTTTGC-3'
800 ug Unpurified and Lyophilized
MW = 13511 ug/umol pmol/ug = 74 %GC = 38.6 Tm = 122°C
Notes: pTLZ

K9110138 Ct-pmpF-RCh/t-Sal 62mer
5'-AGGCAGAGGGTCGACTTAATGGTGATGGTGATGGTGAAAGACCAGAGCTC
CTCCTGCATTGA-3'
907 ug Unpurified and Lyophilized
MW = 19241 ug/umol pmol/ug = 52 %GC = 51.6 Tm = 188°C
Notes: pTLZ

K9110139 Ct-pmpF-RCs/sb-Xba 44mer
5'-AGGCAGAGGTCTAGATTAAAAGACCAGAGCTCCTCCTGCATTGA-3'
725 ug Unpurified and Lyophilized
MW = 13531 ug/umol pmol/ug = 74 %GC = 47.7 Tm = 130°C
Notes:

Continued on Page 10

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Kelly Johnson
Signed

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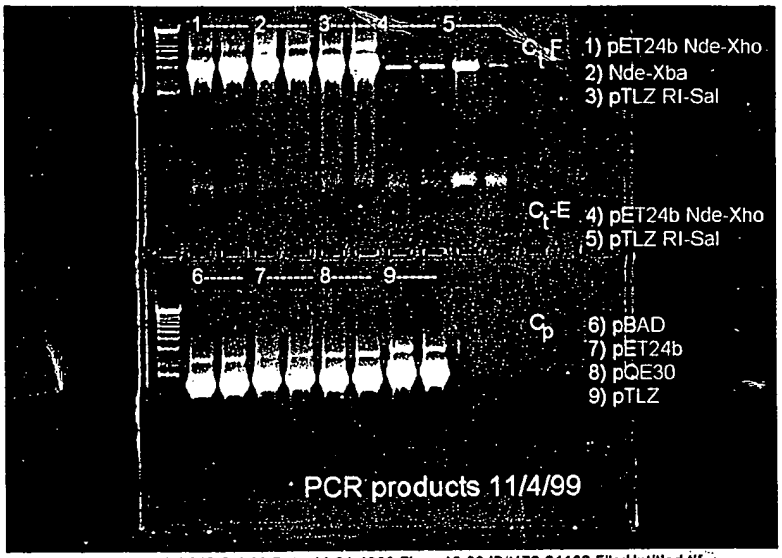
Signed

Date

ESULTS: At right is a photo showing 10 µl of the PCR products from each set, loaded on a 0.8% agarose gel.



Exp:10/30 Sec B:0 W:255 G:1.00 Date:11-04-1999 Time:15:50 ID#472-01160 File:Untitled.tif

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Kelly Johnson
Signed

Date

Signed

Date

EXHIBIT 5

PURPOSE: The purpose of this experiment is to create PCR products for C_t H47 (for pET28a) no his tag and pmpE (for pET28a)

MATERIALS/METHODS: The same method used on page 1 & 2 will be used here as well. The following sets of primers will be used (along with 4 µl C_t genomic DNA)

K9112072 Ct-H47-Fn/28a-Nco' 42mer
5'-ATCCAGCAGAGGCCATGGTGAAAAGATTATTATGTGTGTTGC-3'
1689 ug Unpurified and Lyophilized
MW = 12991 ug/umol pmol/ug = 77 %GC = 42.9 Tm = 120°C
Notes:

L9120747 Ct-H47-RCs/28a-Sal-1 51mer
5'-ATCCAGCAGAGGGCGGACGGCTTACTCGTCTGATTTCAAGACGATGAATC G-3'
1432 ug Unpurified and Lyophilized
MW = 15714 ug/umol pmol/ug = 64 %GC = 52.9 Tm = 156°C
Notes:

L9120748 L2-pmpE-Fm/28a-Nco*-V 42mer
5'-ATCCAGCAGAGGCCATGGTGAAAGCGTTTTTCTTTTTCCTTA-3'
1366 ug Unpurified and Lyophilized
MW = 12844 ug/umol pmol/ug = 78 %GC = 42.9 Tm = 120°C
Notes:

K9110901 L2-pmpE-RCf/28a-Sal 48mer
5'-ATCCAGCAGAGGGTCGACGGCCGAATCGCAGAGCAATTTCCCCATTGA-3'
743 ug Unpurified and Lyophilized
MW = 14725 ug/umol pmol/ug = 68 %GC = 56.3 Tm = 150°C
Notes:

RESULTS: On the following page is a UV photo showing 10 µl of the 4 pcr samples loaded on a 0.8% agarose gel

C ntinued on Pag 21

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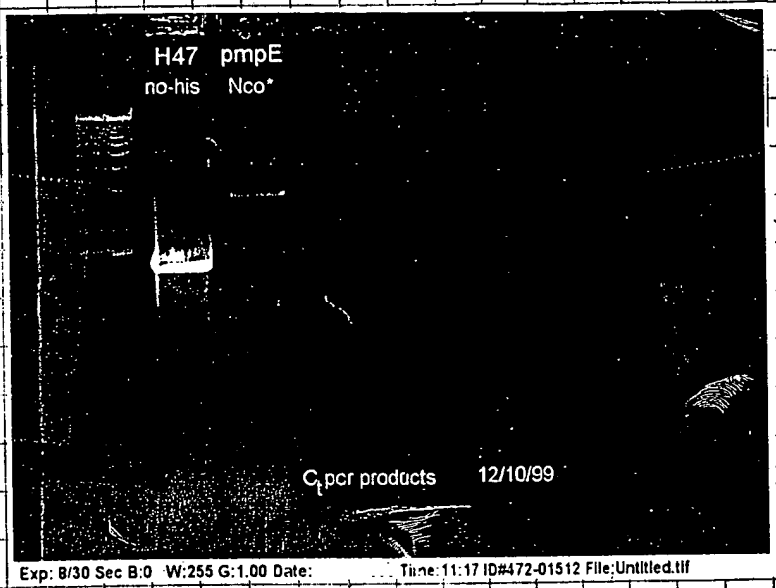
Kelly Johnson
Signed

Date

Signed

Date



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Kelly Johnson
Signed

Date

Andrea D. Harris
Signed

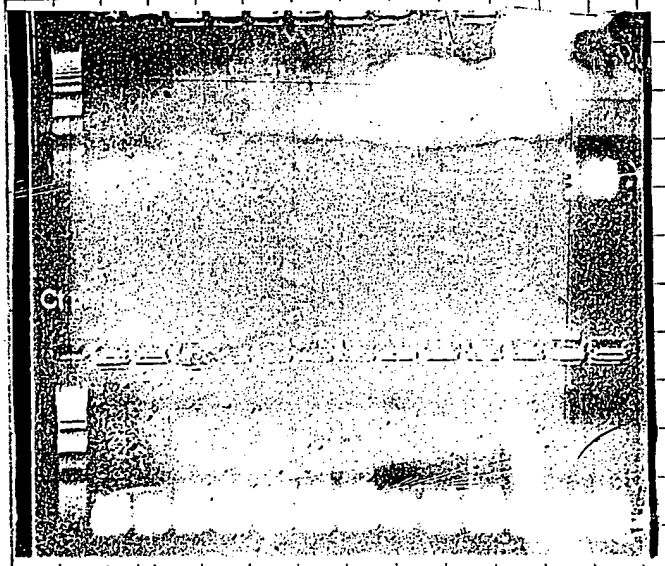
Date

EXHIBIT 6

PURPOSE: The purpose of this experiment is to PCR screen the transformation colonies (Ct pmpE-pQE-M15) for inserts

MATERIALS/METHODS: The same protocol detailed on page 12 will be used here as well

Results: Below are the UV photos showing the 42 samples loaded on a 0.8% agarose gel. #24, #27, #37 appear to be positive.



27 37

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Read and Understood By

Kelly Johnston
Signed

Date

Andrea M. Harris
Signed

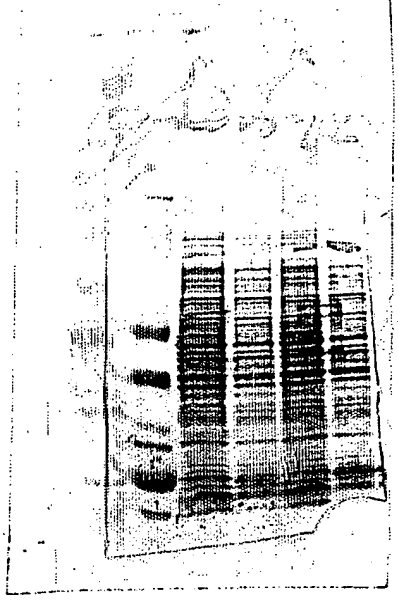
Date

EXHIBIT 7

PURPOSE: The purpose of this experiment is to analyze the positive transformants from CtpmpE/pQE using Western blotting and gel staining after a 3-hour induction

MATERIALS/METHODS: The protocol described on page 14 will be used in this experiment as well

RESULTS: The Western and dried stained gel are below. After the first lane containing the Multi-mark Multicolored Standard the lanes were loaded with a no-induction control, #24 induced, #27 induced, # 37 induced



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Kelly Johnson
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_____
Date

Andria M. Harris
Signed

_____
Date

EXHIBIT 8

PURPOSE: The purpose of this experiment is to do a plasmid prep for the ΔNde + ΔXba pmpE cultures (8 each)

MATERIALS/METHODS: 2 ml of overnight culture was grown and spun down in a microcentrafuge tube @ 14K for 1 minute. The rest of the procedure will be as follows

QIAprep Spin Miniprep Kit Protocol

using a microcentrifuge

This protocol is designed for purification of up to 20 µg of high-copy plasmid DNA from 1–5-ml overnight cultures of *E. coli* in LB (Luria-Bertani) medium. For purification of low-copy plasmids and cosmids, large plasmids (>10 kb), and DNA prepared using other methods, refer to the recommendations on page 31.

! **Please read Important Notes for QIAprep Procedures on pages 14–15 before starting.**

Procedure

1. **Resuspend pelleted bacterial cells in 250 µl of Buffer P1 and transfer to a microfuge tube.**
   Ensure that RNase A has been added to Buffer P1. No cell clumps should be visible after resuspension of the pellet.
2. **Add 250 µl of Buffer P2 and gently invert the tube 4–6 times to mix.**
   Mix gently by inverting the tube. Do not vortex, as this will result in shearing of genomic DNA. If necessary, continue inverting the tube until the solution becomes viscous and slightly clear. Do not allow the lysis reaction to proceed for more than 5 min.
3. **Add 350 µl of Buffer N3 and invert the tube immediately but gently 4–6 times.**
   To avoid localized precipitation, mix the solution gently but thoroughly, immediately after addition of Buffer N3. The solution should become cloudy.
4. **Centrifuge for 10 min.**
   A compact white pellet will form.
   During centrifugation, place a QIAprep spin column in a 2-ml collection tube.
5. **Apply the supernatants from step 4 to the QIAprep column by decanting or pipetting.**
6. **Centrifuge 30–60 sec. Discard the flow-through.**
7. **(Optional): Wash QIAprep spin column by adding 0.5 ml of Buffer PB and centrifuging 30–60 sec. Discard the flow-through.**
   This step is necessary to remove trace nuclease activity when using *endA*+ strains such as the JM series, HB101 and its derivatives, or any wild-type strain, which have high levels of nuclease activity or high carbohydrate content. Host strains such as XL-1 Blue and DH5α™ do not require this additional wash step.
8. **Wash QIAprep spin column by adding 0.75 ml of Buffer PE and centrifuging 30–60 sec.**

The samples will then be eluted with 50µl of dH_2O

Continued on Page 6

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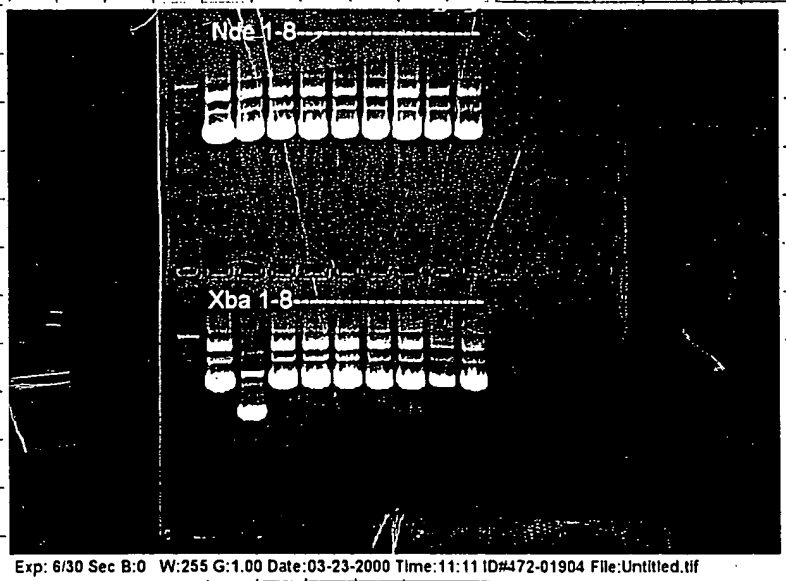
Kelly Johnson — Signed — Date

Andrea Harroo — Signed — Date

RESULTS. Below is a UV photo showing 5µl of the samples loaded on a 0.8% agarose gel.



Exp: 6/30 Sec B:0 W:255 G:1.00 Date:03-23-2000 Time:11:11 ID#472-01904 File:Untitled.tif

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R ad and Underst od By

Kelly Johnson
Signed

Dat

Andrea M. Harris
Signed

Date

PURPOSE: The purpose of this experiment is to sequence the mutagenized Nde plasmid DNA

MATERIALS/METHODS: The protocol supplied by Andrea Harris will be used:

Sequencing protocol:

1. Setting up Reaction:
   - 3 λ DNA 1→5 λ DNA 50 ng → 100 ng/λ
   - 1.7 λ primer 2 pmole/λ
   - 8 λ TRM
   - 7.3 λ H_2O
2. program cycle cycle-sequence ii
3. 20 λ rxn run through sephasose column (rid of excess dNTP's)
   - Centrifuge column @ 3 min 4000 rev.
   - remove column - place in fresh tube
   - line column in same way
   - add sample → to middle of column
   - @ 4 min - 4000 rev.
4. speed-vac - 20 min spin-dry
5. add 25 λ template suppression reagent
   - vortex to resuspend
     - set ~10' vortex hard
   - @ to bring liquid down
6. - Transfer rxn to sequence tube
7. denature 96°C ~~2 min~~ exp.000 cmH
8. @ to bring liquid down slow/short spin
9. Set up machine (ask Andrea)

The sequencing primer that will be used is shown below

K9112070 PmpEsdm-Nde-Seq 25mer
5'-AGTGCAGAACTGACACCTAGTGATC-3'
(1161 ug) Unpurified and Lyophilized
MW = 7651 ug/umol pmol/ug = 131 %GC = 48 Tm = 74°C
Notes:

Once the sequencing reaction and purification are complete the samples will be run using the Abi Prism Genetic Analyzer.

Continued n Pag 68

Read and Understood By

Kelly Johnson Signed _____ Date

Andrea M Harris Signed _____ Date

PROJECT pmpE ΔNde sequencing **Notebook No.** _____

Continued From Page _____

RESULTS: ΔNde samples 4 & 5 were confirmed to have the desired sequence. The data sheets are given below.

ABI PRISM Model 310 Version 2.1.1 Nde-seq-4Sample3/24/0 Signal G:228 A:528 T:354 C:250 DT5%CEHV{A Set-AnyPrimer} Fri, Page 1 of 9:59 AM

Data Collection
File: Nde-seq-4Sample3/24/0
Sample: Nde-seq-4
Lane Number: 4
Number of Scans: 7120
Length: 502
Start Run 24/3/2000, 00:11
Stop Run 24/3/2000, 02:45
Start Collection 24/3/2000, 00:45
Stop Collection 24/3/2000, 02:45
Dyeset/Primer: DTS%CEHV{A Set-AnyPrimer}
Instrument Name: ABI PRISM™ 310
Collect Vers.: ABI PRISM 310 Collection 1.0.4

Data Analysis
Base Call Start: 1224
Base Call End: 7120
Primer Peak Loc.:1224
Signal: G (226), A (528), T (354), C (250)
Matrix Name: 9607067sMATRIX
Analysis Vers.: Version 2.1.1
Base Spacing: 12.05 - ABI-CE1

```
   1  TCCTTTCTGG GGAATTACAG GAGGAGGATA GGCATGATGG TTTACAAGAA TCCTCGAGAA AATCATCCTG GATTCCACAT  80
  81  GCGCTCTTCC GGATACTCTG CGGGGAATGA TAGCAGGGCA AACACATACC TTCTCATTGA AATTCAGTCA GACCTACACC 160
 161  AAACTCAATG AGCGTTACGC GAAAAACAAC GTATCTTCTA AAAATTACTC ATGCCAAGGA GAAATGCTCT TCTCATTGCA 240
 241  AGAAGGTTTC TTGCTGGCTA AATTAGTTGG TCTTTACAGC TATGGAGATC ATAACTGTCA CCATTTCTAT ACCCAAGGAG 320
 321  AAAATCTAAC ATCTCAAGGG ACGTTCCGTA GTCAAACGAT GGGAGGTGCT GTTTTTTTTG ATCTCCCTAT GAAACCCTTT 400
 401  GGATCAACGC ATATACTGAC AGCTCCCTTT TTAGGAGGCA GAGGGAATTC ATGAAAAAAG CGTTTTCTTT TTTCCTTATC 480
 481  GGCAGANGGT CNACTCAATG GG                                                              560
```

ABI PRISM Model 310 Version 2.1.1 Nde-seq-5Sample3/24/0 Signal G:224 A:449 T:191 C:151 DT5%CEHV{A Set-AnyPrimer} Fri, Page 1 of 10:01 AM

Data Collection
File: Nde-seq-5Sample3/24/0
Sample: Nde-seq-5
Lane Number: 5
Number of Scans: 7120
Length: 503
Start Run 24/3/2000, 02:46
Stop Run 24/3/2000, 05:20
Start Collection 24/3/2000, 03:20
Stop Collection 24/3/2000, 05:20
Dyeset/Primer: DTS%CEHV{A Set-AnyPrimer}
Instrument Name: ABI PRISM™ 310
Collect Vers.: ABI PRISM 310 Collection 1.0.4

Data Analysis
Base Call Start: 1229
Base Call End: 7120
Primer Peak Loc.:1229
Signal: G (224), A (449), T (191), C (151)
Matrix Name: 9607067sMATRIX
Analysis Vers.: Version 2.1.1
Base Spacing: 12.34 - ABI-CE1

```
   1  TCNTTTCTGG GGAATTACAG GAGGAGGATA GGCATGATGG TTTACAAGAT CCTCGAGAAA ATCATCCTGG ATTCCACATG  80
  81  CGCTCTTCCG GATACTCTGC GGGGAATGAT AGCAGGGCAA ACACATACCT TCTCATTGAA ATTCAGTCAG ACCTACACCA 160
 161  AACTCAATGA GCGTTACGCG AAAAACAACG TATCTTCTAA AAATTACTCA TGCCAAGGNA GAAATGCTCT TCTCATTGCA 240
 241  AGAAGGTTTC TTGCTGGCTA AATTAGTTGG TCTTTACAGC TATGGAGATC ATAACTGTCA CCATTTCTAT ACCCAAGGAG 320
 321  AAAATCTAAC ATCTCAAGGG ACGTTCCGTA NTCAAACGAT GGGAGGTGCT GTTTTTTTTG ATCTCCCTAT GAAACCCTTT 400
 401  GGATCAACGC ATATACTGAC AGCTCCCTTT TTAGGAGGCA GAGGGAATTC ATGAAAAAAG CGTTTTCTTT TTCCTTATCG 480
 481  GCAGAAGGTC AACTCAATGG GGA                                                              560
```

Continued on Page

Read and Understood By

Kelly Johnson
Signed

Date

Signed

Date

EXHIBIT 9

PURPOSE: The purpose of this experiment is to sequence the mutagenized Nde plasmid DNA

MATERIALS/METHODS: The protocol supplied by Andrea Harris will be used:

Sequencing protocol:

1. Setting up Reaction:
   - 3 λ DNA 1→5 λ DNA 50 ng → 100 ng/λ
   - 1.7 λ primer 2 pmole/λ
   - 8 λ TRM
   - 7.3 λ H_2O
2. program cycle cycle-sequence ii
3. 20 λ sln run through sepharose column (rid of excess dNTP's)
   - Centrifuge column @ 3 min 4000 rev.
   - remove column - place in fresh tube
   - line column in some way
   - add sample → middle of column
   - @ 4 min - 4000 rev.
4. speed-vac - 20 min spin-dry
5. add 25 λ template suppression reagent
   - Vortex to resuspend
   - sit ~10' Vortex hand
   - @ to bring liquid down
6. - Transfer rxn to sequence tube
7. denature 96°C ~~5~~ 2 min exp.000 cmH
8. @ to bring liquid down slow/short spin
9. Set up machine (ask Andrea)

The sequencing primer that will be used is shown below

K9112070 PmpEsdm-Nde-Seq 25mer
5'-AGTGCAGAACTGACACCTAGTGATC-3'
(1161 ug) Unpurified and Lyophilized
MW = 7651 ug/umol pmol/ug = 131 %GC = 48 Tm = 74°C
Notes:

Once the sequencing reaction and purification are complete the samples will be run using the Abi Prism Genetic Analyzer.

Continued on Page 68

Read and Understood By

Kelly Johnson Signed _____ Date

Andrea D. Harris Signed _____ Date

RESULTS: ΔNde samples 4 & 5 were confirmed to have the desired sequence. The data sheets are given below.

ABI PRISM

Model 310 Version 2.1.1 | Nde-seq-4Sample3/24/0 | Signal G:228 A:528 T:354 C:250 DT5%CEHV{A Set-AnyPrimer} | Page 1 of 9:59 AM

Data Collection
File: Nde-seq-4Sample3/24/0
Sample: Nde-seq-4
Lane Number: 4
Number of Scans: 7120
Length: 502
Start Run 24/3/2000, 00:11
Stop Run 24/3/2000, 02:45
Start Collection 24/3/2000, 00:45
Stop Collection 24/3/2000, 02:45
Dyeset/Primer: DT5%CEHV{A Set-AnyPrimer}
Instrument Name: ABI PRISM® 310
Collect Vers.: ABI PRISM 310 Collection 1.0.4

Data Analysis
Base Call Start: 1224
Base Call End: 7120
Primer Peak Loc.:1224
Signal: G (226), A (528), T (354), C (250)
Matrix Name: 9607067sMATRIX
Analysis Vers.: Version 2.1.1
Base Spacing: 12.05 - ABI-CE1

```
   1 TCCTTTCTGG GGAATTACAG GAGGAGGATA GGCATGATGG TTTACAAGAA TCCTCGAGAA AATCATCCTG GATTCCACAT  80
  81 GCGCTCTTCC GGATACTCTG CGGGGAATGA TAGCAGGGCA AACACATACC TTCTCATTGA AATTCAGTCA GACCTACACC 160
 161 AAACTCAATG AGCGTTACGC GAAAAACAAC GTATCTTCTA AAAATTACTC ATGCCAAGGA GAAATGCTCT TCTCATTGCA 240
 241 AGAAGGTTTC TTGCTGGCTA AATTAGTTGG TCTTTACAGC TATGGAGATC ATAACTGTCA CCATTTCTAT ACCCAAGGAG 320
 321 AAAATCTAAC ATCTCAAGGG ACGTTCCGTA GTCAAACGAT GGGAGGTGCT GTTTTTTTTG ATCTCCCTAT GAAACCCTTT 400
 401 GGATCAACGC ATATACTGAC AGCTCCCTTT TTAGGAGGCA GAGGGAATTC ATGAAAAAAG CGTTTTCTTT TTCCTTATC  480
 481 GGCAGANGGT CNACTCAATG GG                                                               560
```

ABI PRISM

Model 310 Version 2.1.1 | Nde-seq-5Sample3/24/0 | Signal G:224 A:449 T:191 C:151 DT5%CEHV{A Set-AnyPrimer} | Page 1 of 10:01 AM

Data Collection
File: Nde-seq-5Sample3/24/0
Sample: Nde-seq-5
Lane Number: 5
Number of Scans: 7120
Length: 503
Start Run 24/3/2000, 02:46
Stop Run 24/3/2000, 05:20
Start Collection 24/3/2000, 03:20
Stop Collection 24/3/2000, 05:20
Dyeset/Primer: DT5%CEHV{A Set-AnyPrimer}
Instrument Name: ABI PRISM® 310
Collect Vers.: ABI PRISM 310 Collection 1.0.4

Data Analysis
Base Call Start: 1229
Base Call End: 7120
Primer Peak Loc.:1229
Signal: G (224), A (449), T (191), C (151)
Matrix Name: 9607067sMATRIX
Analysis Vers.: Version 2.1.1
Base Spacing: 12.34 - ABI-CE1

```
   1 TCNTTTCTGG GGAATTACAG GAGGAGGATA GGCATGATGG TTTACAAGAT CCTCGAGAAA ATCATCCTGG ATTCCACATG  80
  81 CGCTCTTCCG GATACTCTGC GGGGAATGAT AGCAGGGCAA ACACATACCT TCTCATTGAA ATTCAGTCAG ACCTACACCA 160
 161 AACTCAATGA GCGTTACGCG AAAAACAACG TATCTTCTAA AAATTACTCA TGCCAAGGNA GAAATGCTCT TCTCATTGCA 240
 241 AGAAGGTTTC TTGCTGGCTA AATTAGTTGG TCTTTACAGC TATGGAGATC ATAACTGTCA CCATTTCTAT ACCCAAGGAG 320
 321 AAAATCTAAC ATCTCAAGGG ACGTTCCGTA NTCAAACGAT GGGAGGTGCT GTTTTTTTTG ATCTCCCTAT GAAACCCTTT 400
 401 GGATCAACGC ATATACTGAC AGCTCCCTTT TTAGGAGGCA GAGGGAATTC ATGAAAAAAG CGTTTTCTTT TTCCTTATCG 480
 481 GCAGAAGGTC AACTCAATGG GGA                                                               560
```

Continued on Page

Read and Understood By

Kelly Johnson
Signed

Dat

Signed

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EXHIBIT 10

To: Larry Ellingsworth

From: J. Jackson

Date:

Subject: Chlamydia spp.

Project Number: 0120 & 0125

Re: Monthly Report

Personnel: Andrea Harris, Jing Hui Tian, Kelly Johnson

Summary of C.trachomatis pmpE ORF Cloning & Expression:

Efforts to PCR clone and express the C.trachomatis pmpE protein appear to have succeeded. Derivatives of pQE30 have been recently generated that appear to produce low levels of recombinant pmpE protein in shake flasks following IPTG-induction. These derivatives were made such that the mature form of the pmpE (i.e. the coding sequence minus its putative ~25 residue signal sequence) is fused to a short M-RGS N-terminal translantional efficiency domain which precedes a $(His)_6$ affinity purification segment. The sequences of the PCR primers used to clone and express the C.trachomatis protein are presented below.

L2-pmpE-Ff/30-Sal

5' - ATC CAG CAG AG GGT CGA CGG GTT CCA GAT CCT ACG AAA GAG TCG CTA TC - 3' 49 mer

L2-pmpE-RCs/30-Sal

5' - ATC CAG CAG AGG GTC GAC GGC C TTA GAA TCG CAG AGC AAT TTC CCC ATT GA - 3' 51 mer

While a ~100kDa IPTG-inducible protein is not observed in coomassie stained SDS-gels of crude shake flask lysates, a protein of the size expected for pmpE (~100kDa) does react in Western blots employing an anti- $(His)_5$ antibody as probe to detect the N-terminal $(His)_6$ tag. Unlike the recently expressed C.pneumoniae pmp8, pmp9, and pmp13 proteins, several lower molecular weight immunoreactive proteins are also detected in the pmpE blots. This suggests that the pmpE protein is either being degraded to some extent and/or transcription/translation of the pmpE gene is

being prematurely terminated and the termination products are being detected via blotting.

As with the C.pneumoniae pmp8 and pmp9 derivatives, the E.coli M15 pQE30-pmpE #37 expression clone has been transferred to the Fermentation Department for the production of master and working seed banks as well as for the production of cell paste for future purification efforts. Once a fermentation run has been completed with the pQE30-pmpE strain, QC analysis of the resulting material will be needed in order to determine whether the level of "intact" pmpE protein being produced is sufficient enough to warrant subsequent protein purification.

DNA sequence analysis of the pQE30-pmpE insert have just begun to confirm cloning junction sequences and thus verify protein identity. As with the other Chlamydial antigen candidates, a single strand DNA sequence will be derived from the cloned C.trachomatis L_2 pmpE gene to gauge the level of amino acid sequence similarity among different C.trachomatis serovars.

EXHIBIT 11

To: Larry Ellingsworth

From: J. Jackson

Date:

Subject: Chlamydia spp.

Project Number: 0120 & 0125

Re: Monthly Report

Personnel: Andrea Harris, Jing Hui Tian, Kelly Johnson

Ct pmpE:

A single strand DNA sequence for the C.trachomatis pmpE expression clone (Ct-pmpE/pQE #37) has been obtained this month. As expected, analysis of the junction sequences indicate the ~2.9Kbp insert was cloned correctly into pQE30 and no spurious bases were either introduced or deleted during the cloning exercise. Further editing of the single strand sequence will be done as soon as possible to gauge the overall degree of similarity of the L_2 coding sequence to that given on the Berkeley database.

EXHIBIT 12

12-A

C.trachomatis Vaccine Development 1Q Review

Program Goals:

- To develop a vaccine to prevent C.trachomatis infection in sexually active teenagers.

Objectives 2000:

Design a genomic strategy to identify candidate vaccines for evaluation.

Based on computational analyses, initiate studies to clone, express, purify at least two HMW-like proteins and at least two additional high priority candidates and evaluate these antigens in the Tuffery murine infertility model.

EXHIBIT 12

12-B

Percent Identity

	1	2	3	4	5	6	7	8	9		
1		125	120	120					12	1	Pmpg.pro
2	350		27.9	11.4	11.3	12.1	13.1	12.0	12.3	2	Pmpb.pro
3	374.0	101.1		10.6	11.3	11.2	13.0	12.3	12.2	3	Pmpc.pro
4	271.0	299.0	317.0		11.1	11.4	12.7	13.8	12.4	4	Pmpd.pro
5	208.0	332.0	352.0	285.0		15.4	12.2	14.6	13.2	5	Pmpe.pro
6	214.0	386.0	393.0	291.0	158.5		11.8	11.5	12.1	6	Pmpf.pro
7	287.0	344.0	349.0	217.0	335.0	299.0		14.9	14.0	7	Pmpa.pro
8	223.0	371.0	407.0	213.0	253.0	279.0	181.5		12.3	8	Pmpi.pro
9	245.0	349.0	371.0	245.0	296.0	252.0	240.0	262.0		9	Pmph.pro
	1	2	3	4	5	6	7	8	9		

% Identity:

E > I > A > F

E & I

most closely related to HMWP

Divergence:

E > F > I > H

Furthest Related: = pmp C

% Identity 12.0

Divergence 374.0

EXHIBIT 12

12-C

Chlamydia spp. - Cloning & Expression Status

ORF Designation	Lab / BioServe Designation	DNA for PCR	Vector, Resistance	His_6-Tag, Addnl AA	Insert Sequence	ORF Size (bp)	Protein Size (kDa)	Expression Level, Western Blot	Stock Cultures	Ferm.
C.trachomatis Ct-		LGV, L_2								
pmpE	Ct-pmpE/pQE # 37	+	pQE30 / Ap	+ / N-term.	Juncts. OK	2898	104.3	Low / + Multiple forms	+	
C.pneumoniae Cp-										

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to Antex Biologics.

APPENDIX A

Amino acid sequence of insert of Plasmid M15pREP (pQE-Ct-Uni) #37.

MRGSHHHHHHGSACELGTPGRRVPDPTKESLSNKISLTGDTHNLTNCYLDNLRYILAIL
QKTPNEGAAVTITDYLSFFDTQKEGIYFAKNLTPESGGAIGYASPNSPTVEIRDTIGPV
IFENNTCCRPFTSSNPNAAVNKIREGGAIHAQNLYINHNHDVVGFMKNFSYVRGGAIST
ANTFVVSENQSCFLFMDNICIQTNTAGKGGAIYAGTSNSFESNNCDLFFINNACCAGGA
IFSPICSLTGNRGNIVFYNNRCFKNVETASSEASDGGAIKVTTRLDVTGNRGRIFFSDN
ITKNYGGAIYAPVVTLVDNGPTYFINNVANNKGGAIYIDGTSNSKISADRHAIIFNENI
VTNVTSANGTSTSANPPRRNAITVASSSGEILLGAGSSQNLIFYDPIEVSNAGVSVSFN
KEADQTGSVVFSGATVNSADFHQRNLQTKTPAPLTLSNGFLCIEDHAQLTVNRFTQTGG
VVSLGNGAVLSCYKNGAGNSASNASITLKHIGLNLSSILKSGAEIPLLWVEPTNNSNNY
TADTAATFSLSDVKLSLIDDYGNSPYESTDLTHALSSQPMLSISEASDNQLRSDDMDFS
GLNVPHYGWQGLWSWGWAKTQDPEPASSATITDPKKANRFHRTLLLTWLPAGYVPSPKH
RSPLIANTLWGNMLLATESLKNSAELTPSDHPFWGITGGGLGMMVYQEPRENHPGFHMR
SSGYFAGMIAGQTHTFSLKFSQTYTKLNERYAKNNVSSKNYSCQGEMLFSLQEGFLLAK
LVGLYSYGDHNCHHFYTQGENLTSQGTFRSQTMGGAVFFDLPMKPFGSTHILTAPFLGA
LGIYSSLSHFTEVGAYPRSFSTKTPLINVLVPIGVKGSFMNATQRPQAWTVELAYQPVL
YRQELEIATQLLASKGIWFGSGSPSSRHAMSYKISQQTQPLSWLTLHFQYHGFYSSSTF
CNYLNGEIALRF.

Nucleic acid sequence of Plasmid M15pREP (pQE-Ct-Uni) #37.

ATGAGAGGATCGCATCACCATCACCATCACGGATCCGCATGCGAGCTCGGTACCCCGGGTC
GACGGGTTCCAGATCCTACGAAAGAGTCGCTATCAAATAAAATTAGTTTGACAGGAGACAC
TCACAATCTCACTAACTGCTATCTCGATAACCTACGCTACATACTGGCTATTCTACAAAAAA
CTCCCAATGAAGGAGCTGCTGTCACAATAACAGATTACCTAAGCTTTTTTGATACACAAAA
AGAAGGTATTTATTTTGCAAAAAATCTCACCCCTGAAAGTGGTGGTGCGATTGGTTATGCG
AGTCCCAATTCTCCTACCGTGGAGATTCGTGATACAATAGGTCCTGTAATCTTTGAAAATAA
TACTTGTTGCAGACCATTTACATCGAGTAATCCTAATGCAGCTGTTAATAAAATAAGAGAA
GGCGGAGCCATTCATGCTCAAAATCTTTACATAAATCACAATCATGATGTGGTCGGATTTAT
GAAGAACTTTTCTTATGTCCGAGGAGGAGCCATTAGTACCGCTAATACCTTTGTTGTGAGCG
AGAATCAGTCTTGTTTTCTCTTTATGGACAACATCTGTATTCAAACTAATACAGCAGGAAAA
GGTGGCGCTATCTATGCTGGAACGAGCAATTCTTTTGAGAGTAATAACTGCGATCTCTTCTT
TATCAATAACGCCTGTTGTGCAGGAGGAGCGATCTTCTCCCCTATCTGTTCTCTAACAGGAA
ATCGTGGTAACATCGTTTTCTATAACAATCGCTGCTTTAAAAATGTAGAAACAGCTTCTTCA
GAAGCTTCTGATGGAGGAGCAATTAAAGTAACTACTCGCCTAGATGTTACAGGCAATCGTG
GTAGGATCTTTTTTAGTGACAATATCACAAAAAATTATGGCGGAGCTATTTACGCTCCTGTA

GTTACCCTAGTGGATAATGGCCCTACCTACTTTATAAACAATGTCGCCAATAATAAGGGGG
GCGCTATCTATATAGACGGAACCAGCAACTCCAAAATTTCTGCCGACCGCCATGCTATTATT
TTTAATGAAAATATTGTGACTAATGTAACTAGTGCAAATGGTACCAGTACGTCAGCTAATC
CTCCTAGAAGAAATGCAATAACAGTAGCAAGCTCCTCTGGTGAAATTCTATTAGGAGCAGG
GAGTAGCCAAAATTTAATTTTTTATGATCCTATTGAAGTTAGCAATGCAGGGGTCTCTGTGT
CCTTCAATAAGGAAGCTGATCAAACAGGCTCTGTAGTATTTTCAGGAGCTACTGTTAATTCT
GCAGATTTTCATCAACGCAATTTACAAACAAAAACACCTGCACCCCTTACTCTCAGTAATG
GTTTTCTATGTATCGAAGATCATGCTCAGCTTACAGTGAATCGATTCACACAAACTGGGGGT
GTTGTTTCTCTTGGGAATGGAGCAGTTCTGAGTTGCTATAAAAATGGTGCAGGAAATTCTGC
TAGCAATGCCTCTATAACACTGAAGCATATTGGATTGAATCTTTCTTCCATTCTGAAAAGTG
GTGCTGAGATTCCTTTATTGTGGGTAGAGCCTACAAATAACAGCAATAACTATACAGCAGA
TACTGCAGCTACCTTTTCATTAAGTGATGTAAAACTCTCACTCATTGATGACTATGGGAATT
CTCCTTATGAATCCACAGATCTAACCCATGCTCTGTCATCACAGCCTATGCTATCTATTTCT
GAGGCTAGTGATAACCAGCTAAGATCTGATGATATGGATTTCTCGGGACTAAATGTCCCTC
ATTATGGATGGCAAGGACTTTGGAGTTGGGGCTGGGCAAAAACTCAAGATCCAGAACCAG
CATCTTCAGCAACAATCACAGATCCCAAAAAAGCCAATAGATTCCATAGAACCTTATTACT
GACTTGGCTTCCTGCTGGGTATGTTCCTAGCCCGAAACACAGAAGTCCCCTCATAGCGAAT
ACCTTATGGGGGAATATGCTGCTTGCAACAGAAAGCTTAAAAAATAGTGCAGAACTGACAC
CTAGTGATCATCCTTTCTGGGGAATTACAGGAGGAGGACTAGGCATGATGGTTTACCAAGA
ACCTCGAGAAAATCATCCTGGATTCCATATGCGCTCTTCCGGATACTTTGCGGGGATGATA
GCAGGGCAAACACATACCTTCTCATTGAAATTCAGTCAGACCTACACCAAACTCAATGAGC
GTTACGCAAAAAACAACGTATCTTCTAAAAATTACTCATGCCAAGGAGAAATGCTCTTCTC
ATTGCAAGAAGGTTTCTTGCTGGCTAAATTAGTTGGTCTTTACAGCTATGGAGATCATAACT
GTCACCATTTCTATACCCAAGGAGAAAATCTAACATCTCAAGGGACGTTCCGTAGTCAAAC
GATGGGAGGTGCTGTTTTTTTTGATCTCCCTATGAAACCCTTTGGATCAACGCATATACTGA
CAGCTCCCTTTTTAGGTGCTCTTGGTATTTATTCTAGCCTGTCTCACTTTACTGAGGTGGGAG
CCTATCCGCGAAGCTTTTCTACAAAGACTCCTTTGATCAATGTCCTAGTCCCTATTGGAGTT
AAAGGTAGCTTTATGAATGCTACCCAAAGACCTCAAGCCTGGACTGTAGAATTGGCATACC
AACCCGTTCTGTATAGACAAGAACTAGAGATCGCGACCCAGCTCCTAGCCAGTAAAGGTAT
TTGGTTTGGTAGTGGAAGCCCCTCATCGCGTCATGCCATGTCCTATAAAATCTCACAGCAAA
CACAACCTTTGAGTTGGTTAACTCTCCATTTCCAGTATCATGGATTCTACTCCTCTTCAACCT
TCTGTAATTATCTCAATGGGGAAATTGCTCTGCGATTCTAA

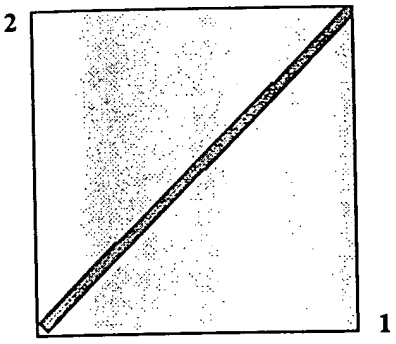
Blast 2 Sequences results

BLAST 2 SEQUENCES RESULTS VERSION BLASTP 2.2.1 [Aug-1-2001]

Matrix: BLOSUM62 gap open: 11 gap extension: 1
x_dropoff: 50 expect: 10.0 wordsize: 3 Filter: ☑ Align

Sequence 1 lcl|seq_1 **Length** 956 (1 .. 956)

Sequence 2 lcl|seq_2 **Length** 965 (1 .. 965)



NOTI ... (bitscore and expect value) is calculated based on the size of nr database

```
Score = 1885 bits (4884), Expect = 0.0
Identities = 925/934 (99%), Positives = 930/934 (99%)

Query: 23  VPDPTKESLSNKISLTGDTHNLTNCYLDNLRYILAILQKTPNEGAAVTITDYLSFFDTQK 82
           VPDPTKESLSNKISLTGDTHNLTNCYLDNLRYILAILQKTPNEGAAVTITDYLSFFDTQK
Sbjct: 32  VPDPTKESLSNKISLTGDTHNLTNCYLDNLRYILAILQKTPNEGAAVTITDYLSFFDTQK 91

Query: 83  EGIYFAKNLTPESGGAIGYASPNSPTVEIRDTIGPVIFENNTCCRPFTSSNPNAAVNKIR 142
           EGIYFAKNLTPESGGAIGYASPNSPTVEIRDTIGPVIFENNTCCRPFTSSNPNAAVNKIR
Sbjct: 92  EGIYFAKNLTPESGGAIGYASPNSPTVEIRDTIGPVIFENNTCCRPFTSSNPNAAVNKIR 151

Query: 143 EGGAIHAQNLYINHNHDVVGFMKNFSYVRGGAISTANTFVVSENQSCFLFMDNICIQTNT 202
           EGGAIHAQNLYINHNHDVVGFMKNFSYVRGGAISTANTFVVSENQSCFLFMDNICIQTNT
Sbjct: 152 EGGAIHAQNLYINHNHDVVGFMKNFSYVRGGAISTANTFVVSENQSCFLFMDNICIQTNT 211

Query: 203 AGKGGAIYAGTSNSFESNNCDLFFINNACCAGGAIFSPICSLTGNRGNIVFYNNRCFKNV 262
           AGKGGAIYAGTSNSFESNNCDLFFINNACCAGGAIFSPICSLTGNRGNIVFYNNRCFKNV
Sbjct: 212 AGKGGAIYAGTSNSFESNNCDLFFINNACCAGGAIFSPICSLTGNRGNIVFYNNRCFKNV 271

Query: 263 ETASSEASDGGAIKVTTRLDVTGNRGRIFFSDNITKNYGGAIYAPVVTLVDNGPTYFINN 322
           ETASSEASDGGAIKVTTRLDVTGNRGRIFFSDNITKNYGGAIYAPVVTLVDNGPTYFINN
Sbjct: 272 ETASSEASDGGAIKVTTRLDVTGNRGRIFFSDNITKNYGGAIYAPVVTLVDNGPTYFINN 331

Query: 323 VANNKGGAIYIDGTSNSKISADRHAIIFNENIVTNVTSANGTSTSANPPRRNAITVASSS 382
           +ANNKGGAIYIDGTSNSKISADRHAIIFNENIVTNVT+ANGTSTSANPPRRNAITVASSS
Sbjct: 332 IANNKGGAIYIDGTSNSKISADRHAIIFNENIVTNVTNANGTSTSANPPRRNAITVASSS 391

Query: 383 GEILLGAGSSQNLIFYDPIEVSNAGVSVSFNKEADQTGSVVFSGATVNSADFHQRNLQTK 442
           GEILLGAGSSQNLIFYDPIEVSNAGVSVSFNKEADQTGSVVFSGATVNSADFHQRNLQTK
Sbjct: 392 GEILLGAGSSQNLIFYDPIEVSNAGVSVSFNKEADQTGSVVFSGATVNSADFHQRNLQTK 451

Query: 443 TPAPLTLSNGFLCIEDHAQLTVNRFTQTGGVVSLGNGAVLSCYKNGAGNSASNASITLKH 502
           TPAPLTLSNGFLCIEDHAQLTVNRFTQTGGVVSLGNGAVLSCYKNGAGNSASNASITLKH
Sbjct: 452 TPAPLTLSNGFLCIEDHAQLTVNRFTQTGGVVSLGNGAVLSCYKNGAGNSASNASITLKH 511

Query: 503 IGLNLSSILKSGAEIPLLWVEPTNNSNNYTADTAATFSLSDVKLSLIDDYGNSPYESTDL 562
           IGLNLSSILKSGAEIPLLWVEPTNNSNNYTADTAATFSLSDVKLSLIDDYGNSPYESTDL
```

```
Sbjct: 512 IGLNLSSILKSGAEIPLLWVEPTNNSNNYTADTAATFSLSDVKLSLIDDYGNSPYESTDL 571

Query: 563 THALSSQPMLSISEASDNQLRSDDMDFSGLNVPHYGWQGLWSWGWAKTQDPEPASSATIT 622
           THALSSQPMLSISEASDNQLRSDDMDFSGLNVPHYGWQGLW+WGWAKTQDPEPASSATIT
Sbjct: 572 THALSSQPMLSISEASDNQLRSDDMDFSGLNVPHYGWQGLWTWGWAKTQDPEPASSATIT 631

Query: 623 DPKKANRFHRTLLLTWLPAGYVPSPKHRSPLIANTLWGNMLLATESLKNSAELTPSDHPF 682
           DP+KANRFHRTLLLTWLPAGYVPSPKHRSPLIANTLWGNMLLATESLKNSAELTPSDHPF
Sbjct: 632 DPQKANRFHRTLLLTWLPAGYVPSPKHRSPLIANTLWGNMLLATESLKNSAELTPSDHPF 691

Query: 683 WGITGGGLGMMVYQEPRENHPGFHMRSSGYFAGMIAGQTHTFSLKFSQTYTKLNERYAKN 742
           WGITGGGLGMMVYQ+PRENHPGFHMRSSGY AGMIAGQTHTFSLKFSQTYTKLNERYAKN
Sbjct: 692 WGITGGGLGMMVYQDPRENHPGFHMRSSGYSAGMIAGQTHTFSLKFSQTYTKLNERYAKN 751

Query: 743 NVSSKNYSCQGEMLFSLQEGFLLAKLVGLYSYGDHNCHHFYTQGENLTSQGTFRSQTMGG 802
           NVSSKNYSCQGEMLFSLQEGFLL KLVGLYSYGDHNCHHFYTQGENLTSQGTFRSQTMGG
Sbjct: 752 NVSSKNYSCQGEMLFSLQEGFLLTKLVGLYSYGDHNCHHFYTQGENLTSQGTFRSQTMGG 811

Query: 803 AVFFDLPMKPFGSTHILTAPFLGALGIYSSLSHFTEVGAYPRSFSTKTPLINVLVPIGVK 862
           AVFFDLPMKPFGSTHILTAPFLGALGIYSSLSHFTEVGAYPRSFSTKTPLINVLVPIGVK
Sbjct: 812 AVFFDLPMKPFGSTHILTAPFLGALGIYSSLSHFTEVGAYPRSFSTKTPLINVLVPIGVK 871

Query: 863 GSFMNATQRPQAWTVELAYQPVLYRQELEIATQLLASKGIWFGSGSPSSRHAMSYKISQQ 922
           GSFMNATQRPQAWTVELAYQPVLYRQE  IATQLLASKGIWFGSGSPSSRHAMSYKISQQ
Sbjct: 872 GSFMNATQRPQAWTVELAYQPVLYRQEPGIATQLLASKGIWFGSGSPSSRHAMSYKISQQ 931

Query: 923 TQPLSWLTLHFQYHGFYSSSTFCNYLNGEIALRF 956
           TQPLSWLTLHFQYHGFYSSSTFCNYLNGEIALRF
Sbjct: 932 TQPLSWLTLHFQYHGFYSSSTFCNYLNGEIALRF 965

CPU time:     0.36 user secs.       0.02 sys. secs          0.38 total secs.

Gapped
Lambda      K       H
   0.316    0.132    0.396

Gapped
Lambda      K       H
   0.267   0.0410    0.140


Matrix: BLOSUM62
Gap Penalties: Existence: 11, Extension: 1
Number of Hits to DB: 12,524
Number of Sequences: 0
Number of extensions: 873
Number of successful extensions: 15
Number of sequences better than 10.0: 1
Number of HSP's better than 10.0 without gapping: 1
Number of HSP's successfully gapped in prelim test: 0
Number of HSP's that attempted gapping in prelim test: 0
Number of HSP's gapped (non-prelim): 1
length of query: 956
length of database: 239,316,239
effective HSP length: 131
effective length of query: 825
effective length of database: 206,523,009
effective search space: 170381482425
effective search space used: 170381482425
T: 9
A: 40
X1: 16 ( 7.3 bits)
X2: 129 (49.7 bits)
X3: 129 (49.7 bits)
S1: 41 (21.6 bits)
S2: 77 (34.3 bits)
```

APPENDIX B

Fertility Assessment for pmpE (FL / GP)

Group	Vaccine & Route	Fertile Females per Total	% Fertility	Number Litters per Total
I	PmpE + AB5 / i.n.	4 / 8	50%	5 / 8
II	PmpE / i.n.	7 / 15	46%	8 / 15
III	AB5 / i.n. (Neg. Control)	2 / 22	9%	3 / 22
IV	AB5 / i.n. (Pos. Control)	19 / 20	95%	41 / 20

Express Mail No.: EL 477 032 898 US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: W. James Jackson

Serial No.: 09/677,752 Group Art Unit: 1645

Filed: October 3, 2000 Examiner: V. Ford

For: CHLAMYDIA PROTEIN, GENE SEQUENCE AND USES THEREOF Attorney Docket No.: 7969-087-999

STATEMENT REGARDING PERMANENCE AND AVAILABILITY OF DEPOSITED MICROORGANISMS

Assistant Commissioner for Patents
U.S. Patent and Trademark Office
P.O. Box 2327
Arlington, VA 22202

Sir:

I, W. James Jackson, declare and state:

1. That I am an authorized Officer of Antex Biologics Inc., the Assignee of the above-identified application.

2. That on September 12, 2000, *E. coli* containing plasmid M15 pREP (pQE-pmpE-Ct) #37 was deposited with the AMERICAN TYPE TISSUE CULTURE COLLECTION (ATCC), at 10801 UNIVERSITY BLVD., MANASSAS, VIRGINIA 20110-2209, USA, International Depository Authority, in compliance with the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure. The deposit was viable at the time of deposit and has been assigned accession number ATCC No. PTA-2462.

3. That I hereby assure the United States Patent and Trademark Office and the public that (a) all restrictions on the availability to the public of a sample of the

deposited microorganism will be irrevocably removed upon issuance of a United States patent of which the microorganism are the subject; (b) the above-mentioned microorganism will be maintained for a period of at least five years after the most recent request for the furnishing of a sample of the deposited microorganism were received by the ATCC and, in any case for a period of at least 30 years after the date of deposit; (c) should the deposited microorganism become non-viable it will be replaced by the Assignee; and (d) access to the deposited microorganism will be available to the Commissioner during the pendency of the patent application or to one determined by the Commissioner to be entitled to such cell line under 37 C.F.R. § 1.14 and 35 U.S.C. § 122.

I hereby declare further that all statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true and further I make these statements with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Respectfully submitted,

Date: 11/29/01

By: _____

W. James Jackson, Vice President
Antex Biologics Inc.